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TUBERCULOUS GUINEA PIGS TO TUBER-
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PHOSPHATIDE

A DISSERTATION SUBMITTED TO THE
GRADUATE FACULTY IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PATHOLOGY
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SION W. HOLLEY

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CORNEAL REACTIONS OF NORMAL AND OF TUBERCULOUS GUINEA PIGS TO TUBERCULO-PROTEIN AND TUBERCULO-PHOSPHATIDE *

SION W. HOLLEY, PH.D.

(From the Department of Pathology, University of Chicago, Chicago, Ill.)

Tissue reactions to the more purified components of the tubercle bacillus indicate that the protein fraction is responsible in a large measure for the acute exudative reaction, including much of the cellular response to the organism, and that the lipoids, particularly the phosphatide, stimulate the more chronic response and particularly the development of epithelioid cells and the lesions characteristic of tuberculosis.

Following intraperitoneal injections of adequate amounts of tuberculo-protein in normal rabbits, Doan, Sabin and Forkner¹ and Miller² observed an outpouring of leukocytes, clasmotocytes, plasma cells and lymphocytes in the omentum. A similar cellular response was found in the meninges of normal and of tuberculous rabbits by Bickford.³ In some of the omentums studied by Miller plasma cells predominated. In general, they were noted by all of the above investigators as a prominent feature of the reactions. Epithelioid cells were never present in significant numbers. After intratracheal injections of tuberculo-protein Larson and Long⁴ observed an exudation of polymorphonuclear and mononuclear leukocytes into the pulmonary alveoli of both normal and tuberculous guinea pigs, the more marked reaction occurring in the latter group. Seibert⁵ noted a slight leukocytic response in the skin of normal guinea pigs following an injection of purified tuberculo-protein. In tuberculous animals and in those sensitized by tuberculo-protein the cellular reaction was marked even to very small amounts of the material; it consisted of many polymorphonuclear neutrophils, large numbers of eosinophiles and mononuclears. Another striking change, as Long⁶ had pointed out previously, was the marked swelling and subsequent degeneration of the connective tissue fibers. In the testes of tuberculous and tuberculo-protein sensitized guinea pigs Seibert found an interstitial edema and infiltration by numerous mononuclear exudate

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cells in response to the protein. In many instances there were polymorphonuclears and eosinophiles also. Some testes showed marked degeneration of the germinal cells. Similar changes had been noted by Long ⁷ after injecting Old Tuberculin into testes of tuberculous guinea pigs. In the later stage of the reaction these organs became atrophic and were infiltrated with large mononuclears. Old Tuberculin was without effect on testes of normal animals. The protein used by Seibert caused a slight leukocytic infiltration in the testes of some normal animals and none in others.

These results have shown that though relatively inert in normal tissues in small amounts, tuberculo-protein in sufficient quantity is capable of stimulating a cellular response somewhat similar to that to the same material in tuberculous animals, except in degree. In the allergic tissues is the added factor of tissue degeneration in many cases.

The biological testing of the various lipoids isolated from the tubercle bacillus has shown that the phosphatide fraction stimulates the most specific cellular reaction. Sabin ⁸ has given a comprehensive review of the literature concerning the activity of the tubercle bacillus lipoids in animal tissues and has summarized the extensive experiments performed by herself and her co-workers with these substances. It was found that repeated injections of the phosphatide into the peritoneal cavities of normal rabbits resulted in the production of masses of epithelioid cells, some of which were arranged to form typical tubercles. Giant cells and caseation were also observed. Injected into the subarachnoid space of normal and of tuberculous rabbits, phosphatide from bovine tubercle bacilli caused a slow production of epithelioid cells.³ On the other hand, Boissevain ⁹ found evidence that tuberculo-phosphatide, if highly purified, did not bring about development of epithelioid cells or tuberculous-like tissue. Such were present when a water-insoluble protein of the tubercle bacillus was used, however. Smithburn and Sabin ¹⁰ found no tuberculous-like tissue after injecting water-insoluble protein derived from tubercle bacilli. Intracutaneously, a rather persistent nodule without any likeness to a tuberculin reaction was produced by tuberculo-phosphatide at the site of injection in tuberculous animals.¹⁰

Since no parallel studies had been made of the action of small amounts of tuberculo-protein and tuberculo-phosphatide in normal and allergic animals, these were undertaken. Because of its access

for continuous observation grossly, and since its avascular structure offered a favorable site for tracing inflammatory cells, the cornea was chosen as the tissue in which to study the reactions to the above materials. Guinea pigs were used. The protein and phosphatide employed were prepared from a human strain of the tubercle bacillus.

MATERIALS

The protein was the purified TPT prepared by Dr. Florence B. Seibert from tubercle bacilli of the H₃₇ strain by the ultrafiltration and trichloroacetic acid method.¹¹ The phosphatide, A₃-a, was also prepared by Dr. Seibert according to the method of Anderson¹² from an alcohol and ether extract of strain 119 tubercle bacilli, which was in fact a strain of H₃₇ from another laboratory. Additional filtrations through Seitz filters were employed for removing remaining bacillary débris. There were no acid-fast particles in the final product, which easily made a permanent fine emulsion in distilled water and in normal saline solution. It contained 0.229 per cent of nitrogen of undetermined nature.

METHOD

A group of male guinea pigs that had been infected for 6 to 8 weeks with H₃₇ tubercle bacilli, and which gave strong tuberculin reactions, received intracorneal injections of approximately 0.01 mg. of tuberculo-protein TPT in 0.005 cc. of normal saline. A group of normal guinea pigs was similarly injected. Another group of tuberculous and one of normal guinea pigs received approximately 0.1 mg. of the tuberculo-phosphatide A₃-a emulsified in 0.005 cc. of normal saline in the cornea. One animal from each group was killed by illuminating gas at 3, 7, 15 and 30 days after injection. At later dates the experiment was repeated twice, with constant results. In one repetition animals were sacrificed at 30 hours and 11 days in addition to the above periods. The injected eyes were removed, fixed in formol-Zenker's solution and prepared for sectioning by the celloidin method. Hematoxylin and eosin stains were used routinely; hematoxylin-eosin azure and a modified Weigert fibrin stain¹³ were used in certain cases.

In the subsequent descriptions the term mononuclear cell will include the various types of uninuclear cells of inflammation except

epithelioid and plasma cells. This is used because of the difficulty in accurately identifying many cells which were of intermediate or transitional types. The cells referred to as epithelioid are mononuclears with a large amount of pale, finely granular cytoplasm and a large vesicular nucleus, their shape being round to elongate, depending apparently on the degree of pressure by adjacent structures. These cells are like those seen in typical tuberculous lesions. The term fibrinoid is used to describe fibrin-like material in the anterior chamber of certain eyes which did not stain like fibrin by special methods for demonstrating this substance.

REACTIONS TO TUBERCULO-PROTEIN

Normal Animals: Grossly at 30 hours there was a slight central cloudiness in the corneas of normal guinea pigs. This diminished rapidly and disappeared by or shortly after the third day.

Microscopically the reaction at 30 hours consisted of numerous polymorphonuclear neutrophils between collagen bundles in a small central area of the cornea, while a few such cells were to be seen in the limbus and migrating toward the center. At 3 days small numbers of cells, a few of them mononuclears, remained, but at subsequent periods the tissue was essentially normal.

Tuberculous Animals: Grossly the cornea of the tuberculous guinea pig sacrificed 30 hours after injection was diffusely grayish white and semitranslucent. At 3 days new capillaries could be seen at the sclerocorneal junction, and the grayish white appearance was more intense, especially 1 to 2 mm. from the corneal margin. By 7 days many vessels were approaching the center, which was now white and almost opaque. Between the 9th and 15th days ulcerations with vascularized margins developed in the opaque area in seven of eight animals. The reaction in the periphery was diminishing. At 30 days all that remained was a poorly demarcated, barely visible, gray, central area traversed by a few small blood vessels.

Microscopically at 30 hours the epithelium was missing from the center of the cornea of the tuberculous guinea pig. This cornea was thickened three to four times normal because of a marked swelling of the collagen bundles and some interstitial edema; the latter was more pronounced in the limbus, where large numbers of polymorphonuclear neutrophils were present in the interstitial spaces. Among them, but more numerous about the vessels than elsewhere, were

many mononuclears of all types from small lymphocytes and other less differentiated small cells to large, round or irregularly shaped cells with large, oval to bilobed nuclei, some containing relatively small amounts of chromatin, others coarse, dark chromatin strands. A few well defined spaces adjacent to blood vessels, perhaps lymphatics since they contained no erythrocytes, were packed with these cells and a few polymorphonuclears. Fibrous connective tissue cells and endothelial cells were swollen. Large numbers of polymorphonuclears had migrated into the cornea proper for a short distance, but few had reached the center, where swelling of the collagenous tissue was the only striking feature. In the anterior chamber, enmeshed in a delicate fibrinoid network, were many polymorphonuclears and an occasional mononuclear. Numerous cells of similar types were in the iris and its angular space.

At 3 days (Fig. 1) masses of polymorphonuclears lay in the interstitial spaces well out in the cornea but were still sparse in the center. They had decreased markedly in the limbus, mononuclears being predominant and increased greatly in number, particularly about the vessels. In some instances they filled well defined perivascular spaces. There was a definite proliferation of new capillaries, in the walls of which endothelial cells in mitosis were found frequently. The lumens of these capillaries contained polymorphonuclears and mononuclears considerably in excess of the normal number, the ratio of the two types being approximately one to one. Examples of migration of these cells through vessel walls were present. The anterior chamber contained many more leukocytes of all types and a more coarse fibrinoid network than previously. There was little change in the iris and the angular spaces. Numerous leukocytes were clustered about the ciliary body.

At 7 days many newly formed capillaries were approaching the center. Throughout the recently vascularized areas mononuclears were numerous and definitely outnumbered polymorphonuclears, while in the non-vascularized center they were still scarce. They predominated over polymorphonuclears in vessels as well, and migration of both types of cells through endothelium was taking place. In the corneal center were large numbers of polymorphonuclears, most of which were degenerating, as indicated by swollen granular cytoplasm and pyknotic nuclei. Some had been phagocytosed by the few large mononuclears present and by other polymorphonuclears.

In one cornea cells of the latter type were confined chiefly to a well localized area, while in all others they were spread diffusely through the tissue. In the limbus, inflammatory cells had diminished and throughout the cornea swelling of collagenous tissue was receding. The inflammatory process was subsiding in the anterior chamber also. Plasma cells in moderate numbers appeared in the iris of one eye.

In the one cornea studied at 11 days the chief change was an extensive ulceration and sloughing of necrotic collagenous tissue and degenerating inflammatory cells in the center. In adjacent areas the cellular constituents were mostly mononuclear, some of which were large with pale cytoplasm and large vesicular nuclei; apparently these were approaching an epithelioid-like state. There was no evidence at this or at earlier periods of local mononuclear cell formation. The few mitotic figures present were of endothelial or of fibroblastic origin as far as could be determined. In the limbus were relatively few cells. Approximately one-third were plasma cells, the remainder mononuclears.

At 15 days typical epithelioid cells and large numbers of more undifferentiated large and small mononuclears, many of them small lymphocytes, were spread diffusely through the corneal center. Whether large mononuclears and epithelioid cells were transformations of the smaller cells could not be positively determined; yet there were many intermediate forms which may well have represented successive stages of development from small mononuclears to the above types. The collagenous tissue in the center was stained lightly and had lost its laminated appearance in places, thus indicating early degeneration. The limbus was little altered from the previous period.

At 30 days the corneas were in various stages of change and recovery. In one the collagenous tissue formed a homogeneous matrix in the center. Here were suspended numerous epithelioid cells, large and small mononuclears, an occasional plasma cell and fibroblasts (Fig. 3). Phagocytosed cell debris was present in some epithelioid cells and in large mononuclears. In adjacent less degenerated areas small mononuclears were predominant. Again, transitional forms toward the epithelioid type were seen frequently. The blood vessels in the center contained fairly numerous, large and small mononuclears and a few polymorphonuclears. As at all previous periods,

some were migrating through vessel walls. Other corneas of this period showed distorted and thinned collagen bundles indicating previous injury and subsequent atrophy. The inflammatory cells had practically disappeared from these.

REACTIONS TO TUBERCULO-PHOSPHATIDE

Normal Animals: Grossly in corneas of normal guinea pigs tuberculo-phosphatide brought about a mild, but persistent, reaction characterized by a gray cloudiness which was confined to the area infiltrated with the phosphatide emulsion. The reaction reached its height at 3 days, then diminished slowly, but was still visible at 30 days.

Microscopically at 30 hours the center of the cornea was infiltrated with a moderate number of polymorphonuclear neutrophils, while in the limbus and migrating from it toward the center were fewer polymorphonuclears and an occasional mononuclear. Most cells of the latter type were about vessels in the limbus. There was no interstitial edema or swelling of collagen bundles. At 3 days a few mononuclears were to be seen in the center, where, however, the reaction still consisted of polymorphonuclears predominantly. At 7 days many of the latter cells were degenerating, and some had been phagocytosed by mononuclears of both large and small types, which had increased in number over the previous period. A very few were migrating from the limbus. In one cornea, in which the reaction was especially marked, perivascular spaces were filled with mononuclears. Some were also in vessel lumens, and a few were passing through the endothelium. By 15 days mononuclears predominated in the center. There were transitional forms between these and epithelioid cells, a few of which were present. At 30 days the latter type of cell was predominant and lay between collagen bundles in the center. They were present in varying number in every cornea receiving the phosphatide, in some cases occupying an area of considerable size. There were no typical tubercles.

Tuberculous Animals: In corneas of tuberculous animals the tuberculo-phosphatide reaction was intense and simulated that to tuberculo-protein in many ways. The marked difference in gross and microscopically was that the phosphatide reaction was localized rather than diffuse. In both normal and tuberculous animals it was more persistent also.

In gross at 30 hours there was a marked, circumscribed, grayish white opacity in the center of the cornea, while the peripheral portions were less involved and blue-gray. The height of the reaction was reached between the 3rd and 7th days. The central opaque area assumed a dense yellowish white color and was even more sharply circumscribed than before, appearing as a spherical nodule set deeply in the corneal tissue. Numerous capillaries that had appeared at the corneal margin by the 3rd day had almost reached the center by the 7th. The peripheral reaction was subsiding at the latter period. Between the 10th and 15th days two of ten corneas developed shallow central ulcers, which persisted but a short time. The dense central inflamed area was small and the vessels less prominent. At 30 days there was only a slight, but localized, central haziness much like that in the corneas of the normal guinea pigs sacrificed at the same period, but more marked than any corresponding corneas receiving the protein.

Microscopically at 30 hours the striking features of the phosphatide reaction in tuberculous guinea pigs were the marked swelling of collagen bundles and the large number of polymorphonuclear leukocytes that filled the interstitial spaces throughout the center of the cornea. The swelling referred to was identical with that described in the tuberculo-protein reaction. The cell types were the same as well. In the limbus, mononuclears and polymorphonuclears were decidedly less numerous than in response to the protein. The same was true of the iris and its angular spaces, and there was almost no involvement of the anterior chamber.

At 3 days (Fig. 2) capillaries were proliferating extensively in the limbus and were pushing into the cornea for short distances. They contained increased numbers of mononuclears and polymorphonuclears in about equal proportions; a few of each type were passing through capillary walls. In the interstitial tissue of the limbus mononuclears showed a slight increase over the earlier period, especially about the blood vessels. In the center was a large, well localized area consisting of polymorphonuclears packed between distorted collagen fibers. Many of the cells showed signs of degeneration. The anterior chamber, iris and angular space were practically devoid of leukocytes, another indication of the definite localization of the reaction to the phosphatide in contrast with that to tuberculo-protein in tuberculous animals.

By 7 days new capillaries had reached the margin of the localized central cell mass, which now contained many mononuclears of all types. Phagocytosis of polymorphonuclears was much in evidence. In the adjacent vascularized tissue mononuclears were predominant; the same was true in the vessels. Between center and limbus as well as in the limbus itself were relatively few inflammatory cells.

In the cornea studied at 11 days the central zone was undergoing necrosis and ulceration. The adjacent vascularized tissue contained large numbers of mononuclears and, as described in the case of the corresponding animal receiving tuberculo-protein, many were approaching an epithelioid-like state. A few mature epithelioid cells were already present.

At 15 days the center was well vascularized, and epithelioid cells were numerous, if not predominant. There were still many mononuclears, especially in lateral portions of the cellular area. Some similar cells were migrating through vessel walls. The swelling of collagen bundles had practically subsided, and the limbus and other parts of the eye were essentially normal except for plasma cells in small numbers.

Thirty days after injection the corneas were identical with those of normal animals receiving phosphatide, except that a few receding vessels were still present in those of the tuberculous animals. There were epithelioid cells (Fig. 4) between collagen fibers in the center of each cornea, comprising a compact mass in some instances, but not arranged as a typical tubercle.

The marked tuberculin-like activity of tuberculo-phosphatide in corneas of tuberculous guinea pigs, in contrast with the mild reaction of normal guinea pigs to the same substance, suggested strongly that it contained tuberculo-protein. The difficulty, if not impossibility, of making an absolute separation of phospholipins and traces of protein is generally recognized, and Wells¹⁴ has called attention to the probability of the presence of some protein in most or all of the so-called lipid antigens of bacterial origin. Since foreign proteins mixed with lipoids often have greater antigenic power than when used alone,¹⁵ it was thought that perhaps the tuberculo-phosphatide may have enhanced the activity of any protein which it might contain. It was suggested that a study of the reactions to tuberculo-protein mixed with a phosphatide other than that from tubercle bacilli be made.

LECITHIN AND TUBERCULO-PROTEIN

Mixtures of 0.1 mg. of emulsified Merck's egg lecithin with 0.0001 mg. and with 0.00001 mg. of tuberculo-protein TPT were injected into the corneas of normal and of tuberculous guinea pigs. The same amounts of these substances were injected separately into corneas of other normal and tuberculous guinea pigs for control. Histological studies were made at 3, 7, 15 and 30 days after injection.

In the normal control animals tuberculo-protein, lecithin, and their mixtures brought about only a minimal reaction of polymorphonuclears and an occasional mononuclear without definite localization. Inflammatory cells were relatively most numerous at 3 days, the corneas being practically normal at subsequent periods. In tuberculous guinea pigs lecithin gave the same result as in normal animals.

There was no constant qualitative or quantitative difference between reactions to the lecithin-protein mixtures and to the equal amounts of tuberculo-protein alone in tuberculous animals. Intensity of the reactions varied with sensitivity of the animals. Some that were cachectic did not react at all; others that were highly sensitive responded almost as intensely and with the same types of cells, swelling of collagen bundles and vascularization of the cornea as did those receiving 0.01 mg. of tuberculo-protein. In most instances the reaction was diffuse, although in a few it was somewhat localized. The same was true of the lecithin-tuberculo-protein mixtures, a few animals injected with them not reacting at all and others strongly, but no more than to the small amounts of tuberculo-protein injected alone. Some reactions were diffuse with many leukocytes in the anterior chamber and limbus; others were confined to the cornea proper.

At 15 days some epithelioid cells and numerous mononuclears were seen in the center of the corneas of those animals responding most markedly, both to lecithin-protein mixtures and to 0.0001 mg. and to 0.00001 mg. of protein alone. The above types of cells were present in appreciable numbers only after vascularization of corneal centers had taken place. Mononuclears in the vessels were more numerous than normally, and some were migrating through the endothelium. As brought out several times previously, epithelioid cells seemed to be the result of transition from mononuclears after

the latter cells had reached the reaction site, chiefly through blood vessels.

In some corneas of the various groups of animals studied at 30 hours and 3 days after injection, a few small groups of bacteria were seen in or near the path of the needle. These organisms appeared morphologically like a micrococcus commonly found in air. They were without any significant effect on the reactions, since in those corneas in which bacteria were present the reactions were the same as those in other corneas of the same groups and periods in which none were found. None were seen later than 3 days after injection, indicating that, if present at first, they had been destroyed in every case after this period, and showing that they did not alter the reactions to the materials used for study.

DISCUSSION

In the above experiments swelling of collagen bundles in corneas of tuberculous guinea pigs receiving tuberculo-protein, also in those receiving tuberculo-phosphatide, was like that described by Seibert ⁵ in the cutaneous reaction of animals sensitive to the protein. The subsequent loss of structure and final partial atrophy of the collagen bundles in the protein reaction demonstrated the marked toxicity of tuberculo-protein for sensitized connective tissue. Cellular response to this material was, except for the presence of epithelioid cells, what one would expect in the reaction of any animal to an antigen with which it had been rendered allergic, namely, polymorphonuclear leukocytes at first, then large numbers of mononuclears. The appearance of epithelioid cells in appreciable numbers in later stages of the reaction was of significance, since the water-soluble protein fraction of tubercle bacilli had not been considered a stimulus for the development of these cells. Their presence in the reaction suggested that some of the epithelioid cells in lesions of tuberculosis may be due directly or indirectly to the action of tuberculo-protein on allergic tissues. Since there was no transformation to the epithelioid state of any of the few mononuclear cells responding to tuberculo-protein in corneas of normal guinea pigs, it would seem that the protein did not act on the mononuclear cells directly, but rather that some factor secondary to the degeneration of collagenous tissue or to degeneration of inflammatory cells brought about the change of

mononuclears to epithelioid cells. The real mechanism of the process is not understood.

The presence of epithelioid cells in later stages of reactions to tuberculo-phosphatide in corneas of both normal and tuberculous guinea pigs gave, without exception, further confirmation of the ability of this fraction of the tubercle bacillus to bring about the production of epithelioid cells, as Sabin and her associates have shown. Another important feature of the reactions was the early localization of responding cells in the center of the cornea, as opposed to the diffuse spreading of cells in tuberculo-protein reactions. The most logical explanation was that the phosphatide, as an emulsion, could not diffuse far from the site of injection because the globules of material could not pass through or between the collagen bundles; hence the central localization of reacting cells. The protein, as a solution, diffused from the cornea into the limbus, iris and anterior chamber, thus calling forth cells into all of these areas.

It seemed correct to assume the presence of tuberculo-protein in the phosphatide in view of its marked tuberculin-like activity in tuberculous animals, also because of its nitrogen content which, though small, might well have been due in part to protein. If this were true, then the localization of the allergic reaction to the phosphatide would indicate that the two substances were closely associated physically or chemically and that, as a result, most of the reaction due to the protein was confined to the area which the phosphatide penetrated at the time of injection. The failure of simple mixtures of lecithin and tuberculo-protein to give a localization of inflammatory cells also pointed to a close association of tuberculo-phosphatide with protein. Whether or not the phosphatide amplified the assumed protein activity by virtue of close association with it has not been determined. Because of the 0.229 per cent of nitrogen in the phosphatide, it is possible that 0.1 mg. injections of the latter contained as much as 0.0015 mg. of protein. This amount might well account for the difference in intensity between tuberculo-phosphatide reactions in normal guinea pigs and tuberculous ones.

In previous experiments^{16, 17} it was shown that most of the mononuclears taking part in reactions to living tubercle bacilli injected into the corneas of guinea pigs and rabbits came into the cornea only after vascularization of it had occurred, and that these cells apparently migrated from the blood vessels. Some of them later differen-

tiated into epithelioid cells. These same observations have been made above in describing the reactions to the tuberculo-protein and phosphatide. It seemed that where there was but a slight irritation, as in the corneas of normal guinea pigs, sufficient numbers of mononuclears were able to migrate from the limbus without the aid of vascularization. Normally the limbus contains small numbers of lymphocytes and other mononuclears to supply some cells for mild injuries. In the presence of severe injuries, however, vascularization was apparently necessary to supply the needed mononuclear cells. Polymorphonuclears were able to migrate in large numbers from the limbus early in the reactions; mononuclears were not. In no case was there evidence of local proliferation of inflammatory cells, there being no mitotic figures except in endothelium and occasionally in cells which seemed surely to be fibroblasts. Neither was amitotic cell division observed. More positive indication of the vascular source of the mononuclears was their increased number in vessel lumens and the observation of some of them migrating through vessel walls. The fact that frequently they were most numerous about vessels was also in favor of their vascular entrance to the site of inflammation.

That epithelioid cells developed from mononuclears after these had reached the area of inflammation seemed to be demonstrated by the absence of epithelioid cells in the lumens of vessels; and the presence in the inflamed tissue of cells apparently in transition from mononuclear to epithelioid form further supported their mononuclear cell origin.

SUMMARY AND CONCLUSIONS

A study of the tissue reactions to purified tuberculo-protein and tuberculo-phosphatide in the corneas of normal and tuberculous guinea pigs was made over a period of 1 month. It was found that tuberculo-protein had a markedly toxic action on the connective tissue of corneas of tuberculous animals and led to inflammation and partial degeneration (tuberculin reaction). Furthermore, it seemed responsible, probably indirectly, for the production of epithelioid cells in the later stages of the allergic reactions. In the amounts used, the protein was practically inert in normal guinea pigs.

Tuberculo-phosphatide also caused an acute tuberculin-like reaction in tuberculous guinea pigs. Inasmuch as the preparation con-

tained a small amount of nitrogen, believed to be an impurity, and not part of the molecule, it was concluded that the reaction noted was probably a reaction to tuberculo-protein. The fact that the unknown substance was closely bound chemically seemed to explain the fact that the acute reaction to the tuberculo-protein alone was diffuse, while that to the tuberculo-phosphatide was localized. In both tuberculous and normal animals epithelioid cells were present at the later periods and persisted longer than did those in most of the tuberculo-protein reactions.

The findings confirmed previous work indicating that in tuberculosis of the cornea most mononuclears taking part in the reactions at the site of injection came from the blood stream, and that epithelioid cells arose from these mononuclears by a process of transition at the site of inflammation.

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DESCRIPTION OF PLATES

PLATE 125

FIG. 1. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-protein. Note marked density in and near limbus due to cellular infiltration, and relatively slight infiltration in center. Note also heavy exudate in anterior chamber. $\times 13.5$.

FIG. 2. Cornea of tuberculous guinea pig 3 days after central injection of tuberculo-phosphatide. Note dense cell "mass" in center and absence of exudate in anterior chamber. $\times 13.5$.



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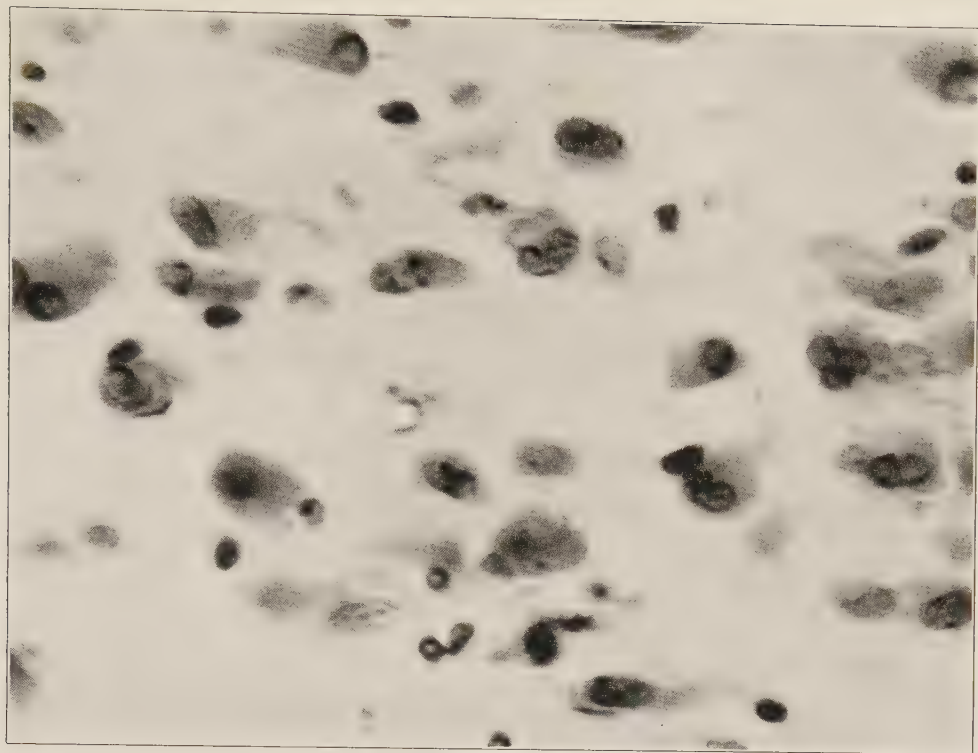


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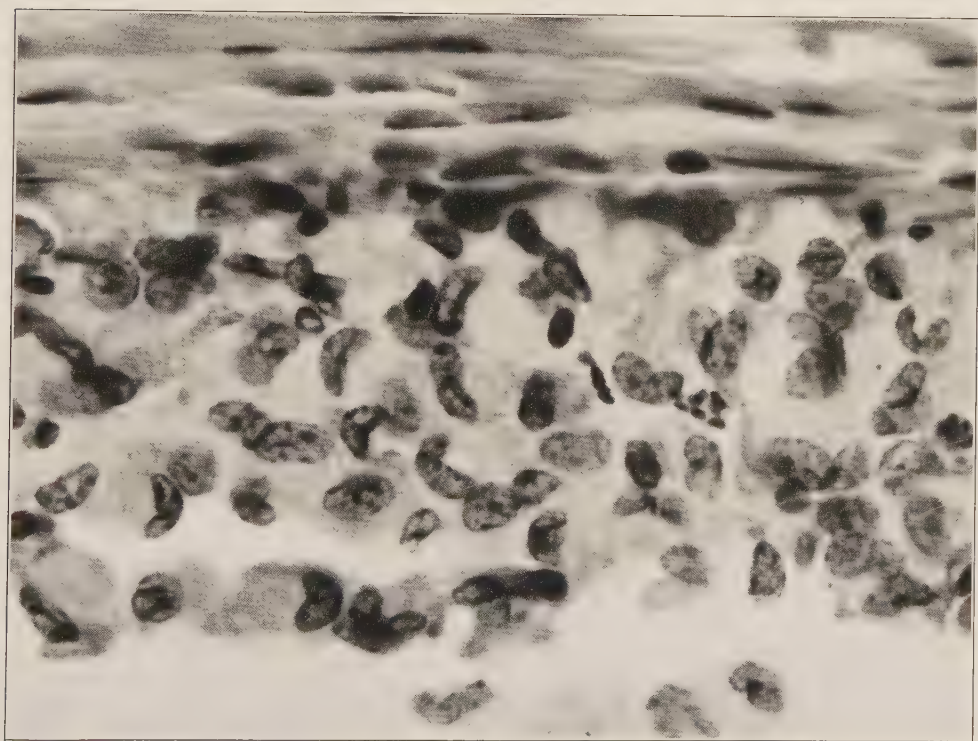
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FIG. 3. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-protein. Note epithelioid and other less differentiated mononuclear cells in homogeneous matrix of degenerated collagenous tissue. $\times 910$.

FIG. 4. Center of cornea of tuberculous guinea pig 30 days after central injection of tuberculo-phosphatide. Note large number of epithelioid cells and interspersed less differentiated mononuclears, some possibly in transition toward epithelioid type. $\times 910$.



3



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